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Running Wheel Accessibility Affects the Regional Electroencephalogram during Sleep in Mice

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Regional aspects of sleep homeostasis were investigated in mice provided with a running wheel for several weeks. Electroencephalogram (EEG) spectra of the primary motor (frontal) and somatosensory cortex (parietal) were recorded for three consecutive days. On a single day (day 2) the wheel was locked to prevent running. Wheel running correlated negatively with the frontal–parietal ratio of slow-wave activity (EEG power between 0.75 and 4.0 Hz) in the first 2 h after sleep onset ($r = -0.60$; $P < 0.01$). On day 2 frontal EEG power (2.25–8.0 Hz) in non-rapid eye movement sleep exceeded the level of the previous day, indicating that the diverse behaviors replacing wheel-running elicited more pronounced regional EEG differences. The frontal–parietal power ratio of the lower frequency bin (0.75–1.0 Hz) in the first 2 h of sleep after dark onset correlated positively with the duration of the preceding waking ($r = 0.64$; $P < 0.001$), whereas the power ratio in the remaining frequencies of the delta band (1.25–4.0 Hz) was unrelated to waking. The data suggest that in mice EEG power in the lower frequency, corresponding to the slow oscillations described in cats and humans, is related to local sleep homeostasis.

Keywords: behavior, EEG spectral analysis, local sleep, regional EEG differences, sleep, sleep homeostasis

Introduction

Slow-wave activity (SWA; EEG power between 0.75 and 4.0 Hz; delta band) in non-rapid eye movement (NREM) sleep increases proportionally to the duration of preceding waking and is considered a measure for sleep intensity (Borbély, 1982; Tobler and Borbély, 1986). The level of SWA differs between cortical areas, suggesting that local mechanisms are involved in sleep regulation. Thus, increased sleep propensity, e.g. after sleep deprivation (SD), leads to a higher initial level of SWA in frontal regions during sleep in humans and several rodent species (Werth *et al.*, 1996; Cajochen *et al.*, 1999; Finelli *et al.*, 2001; Schwierin *et al.*, 1999; Huber *et al.*, 2000b, 2002; Palchykova *et al.*, 2002; Vyazovskiy *et al.*, 2002, 2004b). It has been proposed that the regional differences in sleep intensity may be a consequence of changes in neuronal activation during wakefulness (Krueger *et al.*, 1999), and that synaptic downscaling occurring during sleep selectively favors strengthening of those synaptic connections that were used most during preceding wakefulness (Tononi and Cirelli, 2003). The interhemispheric asymmetry in SWA induced by unilateral sensory stimulation during prior waking (Kattler *et al.*, 1994; Vyazovskiy *et al.*, 2000, 2004b) supports these notions. In a recent study a motor learning task induced a regional increase of SWA during sleep in human subjects that may have been elicited by task-specific changes in neuronal plasticity (Huber *et al.*, 2004).

The mechanisms underlying the frontal predominance of SWA during sleep in rodents are unknown and could be both morphological and/or functional. Thus, the hyperfrontality of SWA may arise from a higher synaptic density, and a larger synaptic weight in the frontal cortical areas. However, it is known for the rat that there are less neurons in the frontal cortex than in the posterior areas (Beaulieu, 1993). On the other hand, frontal areas receive a massive input from the contralateral cortical areas via the corpus callosum (Ozaki and Wahlsten, 1993), from the thalamus (Arbuthnott *et al.*, 1990) and from the brainstem (Lamour *et al.*, 1982; Woolf *et al.*, 1983). Therefore, the relative number of synaptic connections formed in the frontal cortex may be higher compared with other areas.

Support for a functional aspect of the frontal predominance of SWA can be obtained by clarifying whether it is affected by the duration of waking, and whether a use-dependent process contributes to the differences in SWA between cortical regions. In humans frontal EEG power in NREM sleep below 1 Hz was correlated with neuropsychological performance and waking EEG activity in the theta band (Anderson and Horne, 2003a,b). In animals there is some evidence that different types of behaviors activate specific neuronal networks. Thus, adult rats performing a motor task showed increased synaptogenesis and long-term potentiation in the motor area of the neocortex (Rioult-Pedotti *et al.*, 1998, 2000). Similarly, in rats and mice running-wheel (RW) activity enhanced cell proliferation and angiogenesis in motor areas of the neocortex (Kleim *et al.*, 2002; Swain *et al.*, 2003; Ehninger and Kempermann, 2003). However, in mice the level of fos expression in the prefrontal and medial frontal cortex did not correlate with the distance run in a RW, but instead was up-regulated in both regions only after wheel-running was prevented (Rhodes *et al.*, 2003).

The aim of the present study was to make use of the spontaneous tendency of rodents to run when they are provided with a RW (e.g. Hanagasioglu and Borbély, 1982; Sherwin, 1998; Lancel *et al.*, 2003). After prolonged adaptation of C57BL/6 mice to a RW we expected the long running episodes to elicit a homeostatic increase of SWA and a change in EEG topography. Moreover, we predicted that blocking the wheel for a day would elicit a marked diversity of other active behaviors, leading to changes in the EEG spectra of a (frontal) primary motor area and a (parietal) somatosensory area.

Materials and Methods

Animals

The experiment was approved by the Veterinary Office of the Canton of Zurich. Male C57BL/6 mice (Harlan, The Netherlands, $n = 9$), 15.5 ± 0.4 (mean ± SEM) weeks old, weight 25.5 ± 0.6 g at surgery were used. Throughout the experiment, the mice were kept individually in

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Macrolon cages (36 × 20 × 35 cm), equipped with RWs, with food and water available *ad libitum*, and maintained on a 12 h light–12 h dark cycle (daylight type fluorescent tubes, 58 W, ~30 lx) at 22–24°C ambient temperature. RW dimensions were: diameter, 15 cm; regularly spaced PVC bars, length 5 cm, diameter 3 mm; distance between bars, 4 mm; one revolution = 47.1 cm). RW- and passive infra-red (IR) activity were recorded continuously throughout the experiment. All mice were well adapted to using the RW by the time of surgery. The IR sensor placed above the cage generated a signal (activity count) in response to the spatial movement of the animal, detected by displacement of the heat emanating from the animal. RW-revolutions and activity counts were integrated over consecutive 1 min epochs and stored on a computer, as described previously (Tobler *et al.*, 1996; Deboer and Tobler, 2000; Chronobiology Kit, Standford Software System, Stanford, CA).

Surgery

Under deep anesthesia (87 mg/kg ketamin and 13 mg/kg xylazin i.p.; Tobler *et al.*, 1997; Vyazovskiy *et al.*, 2005), epidural electrodes for recording the EEG and two gold wires to record the nuchal muscle EMG were implanted. Stainless steel electrodes (0–80 × 1/8, Plastics One® Inc., Roanoke, VA) were placed over the left and right frontal cortex above the M1 region (2 mm anterior to bregma, 1.5 lateral to the midline; Paxinos and Frankin, 2001), over the left and right parietal cortex (3 mm posterior to bregma, 2 mm lateral to midline) and, as a reference, over the cerebellum. The electrodes were connected to stainless steel wires that were fixed to the skull with dental cement. At least 12 days were allowed for recovery after surgery and adaptation to the recording conditions. The mice were provided with a RW for 37.8 ± 3.4 days prior to the sleep recordings.

Experimental Protocol and Data Acquisition

After surgery the mice were connected by a fine cable, which interfered only minimally with their voluntary behaviour, to the amplifier via a swivel. All mice resumed RW activity within several days after surgery. Upon recovery from surgery (at least 12 days), the EEG and EMG were recorded continuously for three consecutive days (referred to as experimental days 1, 2 and 3), starting at dark onset. Experimental day 1 consisted of an undisturbed 24 h baseline, during which the mice continued to use the RW *ad libitum*. On day 2 the RW was locked 5 min prior to dark onset to prevent running for the next 24 h whilst still allowing access to the wheel. On day 3, at dark onset, the animals were again allowed to run in the wheel (note some running activity in the last 5 min before dark onset in Fig. 1*b*).

The EEG and EMG were recorded with a portable recording system (Institute of Pharmacology and Toxicology, Zurich, Switzerland). Before each recording a calibration signal (10 Hz sine wave, 300 μV peak to peak) was recorded on the EEG and EMG channel. Both signals were amplified (amplification factor ~2000), conditioned by analog filters (high-pass filter –3 dB at 0.16 Hz) and sampled at 512 Hz. The signals were filtered by a digital Finite Impulse Response filter: EEG: low-pass filter: 0 dB at 30 Hz; EMG: band-pass filter: 0 dB at 20–40 Hz. EEG power spectra were computed for 4-s epochs as described previously (Tobler *et al.*, 1997). All epochs were visually inspected and artifacts were always removed simultaneously from both derivations (6.9 ± 2.4% of total recording time, 81.1 ± 3.6% of all artifacts occurred during waking).

Vigilance States

The vigilance states (waking, NREM sleep and REM sleep) were scored off-line for 4 s epochs by visual inspection of the raw parietal EEG signal and the EMG as well as EEG power in the slow-wave range (0.75–4.0 Hz) (see Tobler *et al.*, 1997). Sleep latency was defined as time elapsed between dark onset and the first episode containing at least 5 min of sleep (NREM + REM sleep), whereby interruptions by brief awakenings of <16 s were allowed.

Statistics and Analysis

The comparison of EEG spectra and RW activity between days, EEG spectra between derivations, and the time course of the EEG spectrum after sleep onset was performed by one- or two-way analyses of variance (ANOVAs) with factor 'day' or 'derivation' or 'time-interval'. Whenever significant effects were present ($P < 0.05$) two-tailed paired *t*-tests were used to further evaluate differences. SAS (SAS Institute, Inc., Cary, NC, USA) and MATLAB (The Math Works, Inc., Natick, MA) were used for statistical analysis. The relationship between sleep, as well as the sleep EEG in the first 2 h interval after sleep onset in the dark period, and previous waking duration, or intensity of wheel-running was assessed by linear correlation analyses. For several computations SWA was subdivided into the low frequency bin (0.75–1.0 Hz) and higher frequency delta activity (1.25–4.0 Hz). We investigated also whether general activity during waking, as reflected by the mean number of IR counts per minute and mean EMG activity (EMG variance) per 4 s epoch, has an effect on subsequent sleep. These correlations were computed within

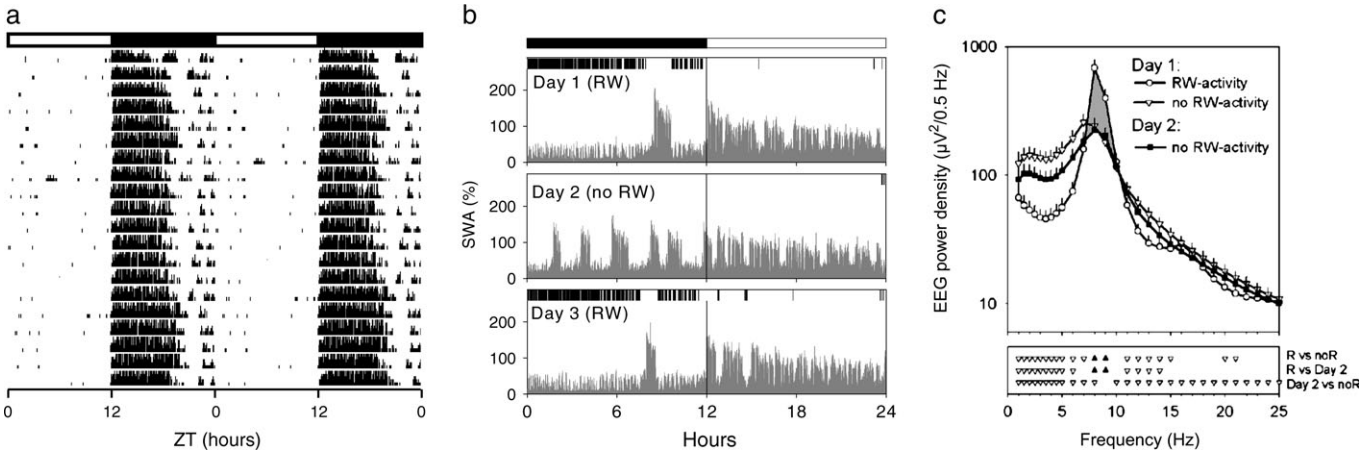

Figure 1. (*a*) Running wheel (RW) activity recorded for three weeks in a representative C57BL/6 mouse. The activity record is double-plotted to better visualize the daily activity pattern at the onset of both the light and dark phases (the *x*-axis represents two consecutive days; the second day is always illustrated twice, once on the right side and once under the previous day, on the left). It shows the percentile distribution of daily RW activity for 21 days. ZT (Zeitgeber): time (h) relative to the entraining agent, the light–dark cycle (dark onset = ZT 12). (*b*) RW activity (black bars within the panels) and relative SWA (EEG power between 0.75 and 4.0 Hz, % of 3-d mean SWA in NRE01 sleep) for 3 days (day 1 and day 3: running *ad libitum*; day 2: RW is blocked). (*c*) EEG power density in waking during the 12 h dark periods of day 1 (RW) and 2 (no RW). Curves connect mean values ($n = 9$) of absolute EEG power density in the parietal derivation for 1 min epochs with and without RW activity. Values are plotted at the upper limit of each frequency bin. Lower panel: differences between bins with RW activity versus bins without RW activity on day 1 (R versus noR) and day 2 (R versus day 2) and differences between epochs without RW activity on days 1 and 2 (day 2 versus noR; $P < 0.05$, paired *t*-test on log-transformed spectral values). Orientation of triangles indicates the direction of deviation. Filled triangles show higher theta power during running.

day 2 only (no RW), since on days 1 and 2 both IR and EMG activity would be largely determined by the running in the wheel.

Results

Access to a Running Wheel Induces a Prolonged Period of Wakefulness in the Dark Period

Access to the RW led to vigorous running (Fig. 1*a*; mean distance in km on day 1 = 11.8 ± 1.4; on day 3 = 14.4 ± 2.1) that resulted in a significant increase of sleep latency (Table 1, Fig. 1*b*). When the RW was locked on day 2, sleep latency after dark onset was markedly shortened from 9 h on day 1 to 4 h on day 2, with a concomitant increase in sleep duration (Table 1). EEG power density during running was characterized by a pronounced peak at 8 Hz in both derivations (Fig. 1*c*, gray area and black triangles). Within the theta frequency range (7.25–9 Hz) power decreased significantly when the animals were not running, whereas power below and above this frequency band was enhanced. On day 2, IR activity was lower than on the other two days (Table 1). However, the relatively high IR activity values indicated a considerable amount of active behavior, accompanied with significantly increased EEG power in the theta band (7.25–12 Hz) compared with epochs with no IR activity (not shown). The individual 24 h IR counts on day 2 varied between 3819–9603. Waking EMG activity on day 2 was lower than on days 1 and 3 (% of the 3 day mean: day 1 = 114.4 ± 2.7; day 2 = 73.9 ± 3.9; day 3 = 109.9 ± 2.0, paired *t*-test, $P < 0.001$).

The Running Wheel Block Elicited Regional Differences in the NREM Sleep EEG

Consistent regional differences between the days were evident in the EEG, especially in high delta and theta frequencies. When the RW was locked on day 2, an increase of EEG power in NREM sleep above the level of day 1 was observed in frequencies between 2.25–8 Hz in the frontal derivation and a concomitant decrease in power in a broad frequency range in the parietal derivation (Fig. 2, left panel). Since wheel-running occurred almost exclusively during the dark period, EEG spectra in NREM sleep were computed separately for the 12 h dark and 12 h light period (Fig. 3). A more pronounced frontal predominance in power occurred on day 2 in frequencies between 3.75 and 6 Hz during the dark period and 2.25 and 6 Hz during the light period, as well as in a broad range above 9 Hz. The frontal predominance had diminished in the light period of both days (Fig. 3). On day 3, when mice again could run in the wheel, the spectra of both derivations and the ratio between them no longer differed from day 1, except in single, scattered bins (Fig. 2, right panel). The intensity of running on the days with RW access showed a negative correlation ($r = -0.60$; $P < 0.01$) with frontal predominance (frontal–parietal ratio) of SWA in

Table 1
Vigilance states, wheel-running and locomotor activity

Day	LD	Waking (h)	NREMS (h)	REMS (h)	Sleep latency (h)	RW (revolutions)	IR (counts)
1	12 h D	10.7 (0.2)	1.2 (0.2)	0.14 (0.02)	8.9 (0.6)	24987.4 (2908.6)	12285.1 (3582.7)
	12 h L	3.8 (0.2)	6.9 (0.2)	1.36 (0.03)		151.1 (86.6)	469.6 (65.9)
2	12 h D	9.1 (0.3)[a]	2.5 (0.3)[a]	0.37 (0.03)[a]	3.8 (0.9)[a]	–	5520.4 (651.7)
	12 h L	4.4 (0.2)[a]	6.3 (0.2)[a]	1.38 (0.04)		250.2 (59.9)	843.3 (155.1)[a]
3	12 h D	11.0 (0.2)[bc]	0.9 (0.2)[bc]	0.08 (0.02)[bc]	9.5 (0.7)[c]	30252.8 (4347.8)[bc]	14349.4 (3863.4)[c]
	12 h L	3.8 (0.2)[c]	6.9 (0.2)[c]	1.36 (0.05)		370.9 (180.2)	654.7 (167.3)

Mean values (SEM in parentheses, $n = 9$) in hours of waking, NREM sleep (NREMS) and REM sleep (REMS), sleep latency (time before the first consolidated sleep episode of the dark period lasting at least 5 min), and amount of running wheel (RW, total number of revolutions) and locomotor activity (passive-infra red counts, IR). Values are for the 12 h light (L) and dark (D) periods of day 1 (RW), day 2 (no RW) and day 3 (RW). Differences between days: [a]day 1 versus day 2; [b]day 1 versus day 3; [c]day 2 versus day 3, $P < 0.05$, paired *t*-test.

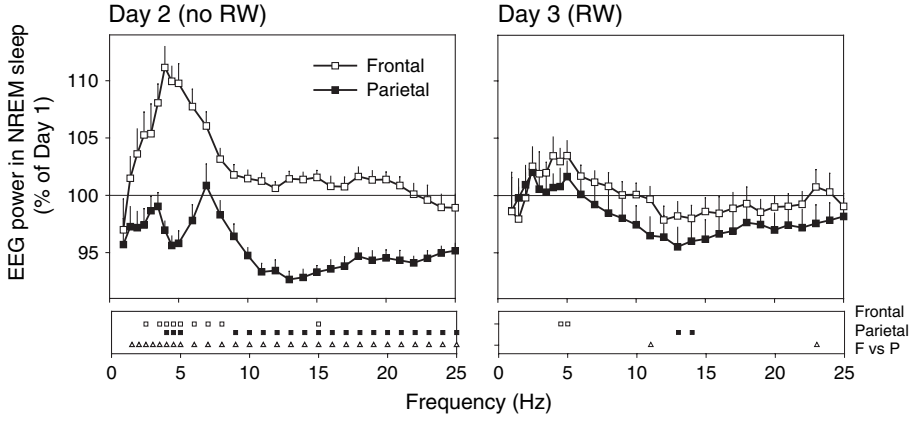

Figure 2. Daily EEG power density in non-REM (NREM) sleep for the frontal and parietal derivation on day 2 (no RW) and day 3 (RW); both days expressed relative to day 1 (RW). Mean values ($n = 9$) of relative power density of each bin are expressed as a percentage of the 24 h mean value in NREM sleep of the same bin on day 1. Squares in the lower panels represent frequency bins where EEG power differed significantly from day 1. Significant differences between the frontal and parietal derivation are indicated by triangles ($P < 0.05$, paired *t*-test). Orientation of triangles indicates the direction of deviation from parietal power.

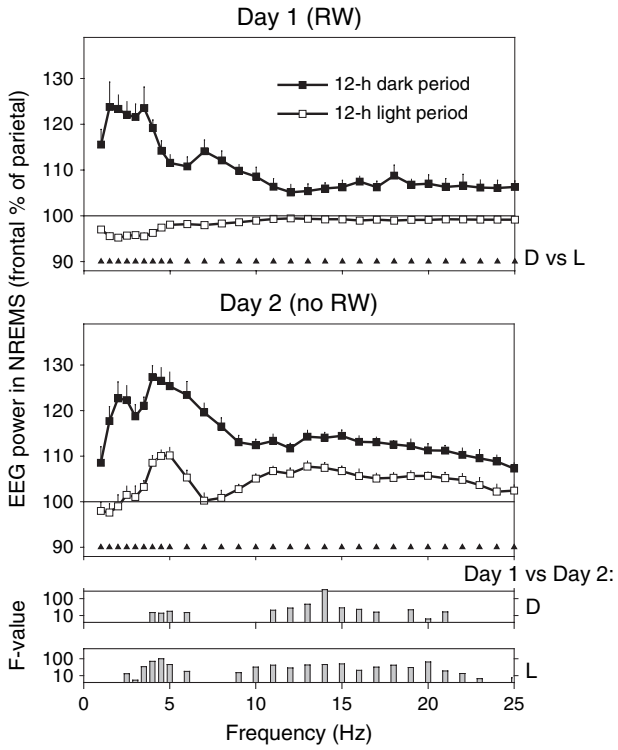

Figure 3. Frontal–parietal ratio of EEG power in NREM sleep of the 12 h intervals (12 h light, 12 h dark period) of days 1 and 2 ($n = 9$). Curves connect mean values of power density expressed as a percentage of the same bin computed for 24 h of day 1. Significant differences between the light and dark period within a day are indicated by triangles ($P < 0.05$, paired t-test). Bars in the bottom panels: F-values for the comparison between the two days ($P < 0.05$; one-way ANOVAs for repeated measures, factors 'day' separately for the dark and light phases).

NREM sleep in the first 2 h after sleep onset. Thus, the highest frontal predominance was associated with the lowest amount of RW activity.

Prolonged Running Increases the Amount of NREM Sleep and SWA in NREM Sleep

After the long waking episode in the dark period of days 1 and 3, power density in NREM sleep was enhanced in frequencies below 6 Hz (parietal EEG) or 11 Hz (frontal EEG), and gradually returned to the average 24 h level (Fig. 4 illustrates day 1). The homeostatic aspect of sleep regulation after the prolonged waking episodes with wheel-running was evident in the significant, positive correlation of both NREM sleep and SWA computed for the first 2 h after sleep onset with the amount of preceding wakefulness between dark onset and the onset of sleep (Fig. 5; NREM sleep in the first 2 h: day 1 = 61.1 ± 4.6 min; day 2 = 42.5 ± 5.5 min; day 3 = 58.8 ± 6.5 min).

On the other hand, the intensity of running correlated only weakly with subsequent SWA and was restricted to the parietal, somatosensory derivation ($r = 0.51$, $P < 0.05$). The correlation was not significant for the frontal derivation ($r = -0.14$) or the amount of NREM sleep ($r = 0.36$). Therefore, neither NREM sleep duration nor the EEG spectrum of the motor cortex was affected by the intensity of running. To investigate whether general activity during waking has an effect on subsequent sleep, separate correlations were computed between EMG or IR activity and the amount of NREM sleep or SWA (or its subdivision) during the first 2 h after sleep onset of day 2. A significant negative correlation was found between EMG activity and parietal EEG power in the low frequency bin (0.75–1.0 Hz, $r = -0.67$, $P = 0.05$), whereas IR activity during waking did not correlate with SWA in subsequent sleep ($P > 0.64$).

Regional Differences in Slow and Fast Delta Power

To investigate whether the regional differences were affected by the wheel use, the NREM sleep EEG spectra of the two derivations were compared (Fig. 6). On all days, power of the frontal EEG exceeded the levels of the parietal EEG in the first 2 h after sleep onset in the dark period (Figs 4 and 6). The regional difference subsided in the course of the next 4 h, reflecting a recovery process (not shown). Despite the frontal predominance in a broad frequency band on days 1 and 2, the 0.75–1.0 Hz bin on day 2 was an exception. Frontal power in this bin remained at the same level as parietal power (Fig. 6, lower panels). The right panel at the bottom of Figure 6 shows that the regional difference in the 0.75–1.0 Hz bin was restricted to days 1 and 3, when running in the wheel was allowed, while the frontal predominance of the remaining frequency bins of the delta band (1.25–4.0 Hz) was similar between the three days.

To determine whether the different regional response of the lowest frequency bin (0.75–1.0 Hz) and the remaining frequencies of the delta band (1.25–4.0 Hz) is related to the amount of waking or, alternatively, to the intensity of running, separate correlations were computed for the two frequency bands. A positive correlation between the duration of waking and the frontal–parietal power ratio during sleep in the subsequent 2 h was restricted to the 0.75–1.0 Hz bin (Fig. 7, top left panel). However, within each derivation, the amount of waking correlated significantly with power both in the 0.75–1.0 Hz bin (parietal: $r = 0.82$; frontal: $r = 0.92$, $P < 0.001$) and in the 1.25–4.0 Hz band (parietal: $r = 0.74$; frontal, $r = 0.48$; $P < 0.05$, not shown). In contrast to the positive relation between the duration of preceding waking, the intensity of wheel-running correlated negatively with the frontal–parietal ratio of SWA (0.75–4.0 Hz; $r = -0.60$, $P < 0.01$). Moreover, after subdivision into the two frequency bands the direction was similar ($r = -0.58$, $P < 0.05$ for 1.25–4.0 Hz and a trend for 0.75–1.0 Hz; Fig. 7, right panels), indicating that a larger frontal predominance of SWA is associated with less running.

Discussion

Previous studies tested the use-dependent hypothesis of sleep regulation by applying selective peripheral activation of the sensory cortex, e.g. vibratory stimulation of the dominant hand in humans (Kattler *et al.*, 1994), unilateral whisker stimulation in an enriched environment in rats and mice (Vyazovskiy *et al.*, 2000, 2004b), or by exploiting a motor learning task in human subjects (Huber *et al.*, 2004). The present study is the first to investigate the effect of preventing wheel-running on regional topography of the sleep EEG. With this manipulation, a motor deprivation was achieved that is evident from the negligible amount of wheel revolutions and the reduced amount of IR activity counts and EMG activity. Thus, depriving mice of a RW provided a powerful approach to investigate the regional EEG changes elicited by the deprivation of vigorous running activity. The distance the mice ran within 24 h was considerable, varying from 7.1 to 25.4 km. We expected that the prevention of running on day 2 would induce differences between the regions,

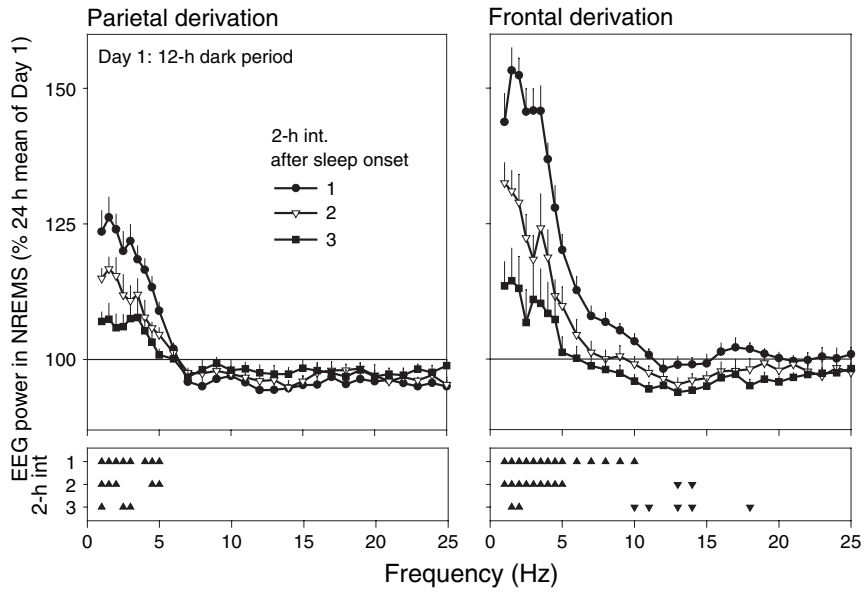

Figure 4. Time course of EEG power density in NREM sleep for the first three intervals after dark onset. Data are shown for the parietal and frontal derivation of the right hemisphere. Mean values of power density for three consecutive 2 h intervals (2 h intervals are based on individually determined sleep onset; $n = 9$) are expressed relative to the same bin of the 24 h mean spectrum (= 100%). Frequency bins which differed from the mean 24 h spectrum of the corresponding derivation are indicated by triangles (paired *t*-test, $P < 0.05$ after significance in one-way ANOVA for repeated measures, factor '2 h interval'). Orientation of triangles indicates the direction of deviation from the 24-h mean of day 1.

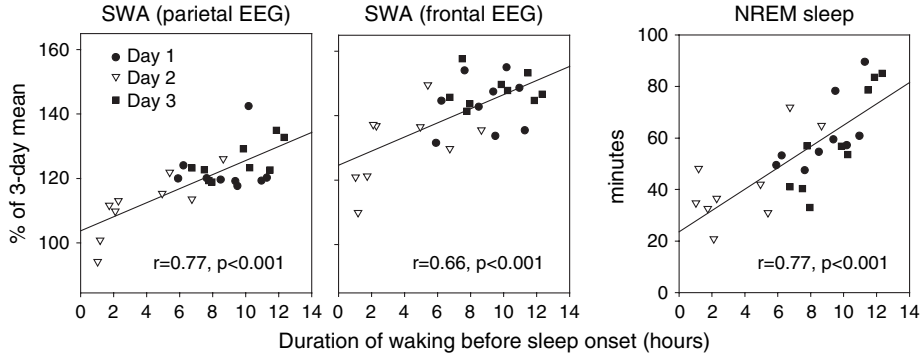

Figure 5. Scatter plots of the correlation between the duration of waking between dark onset and the onset of sleep on days 1, 2 and 3 and SWA (% of 3 day mean) in the parietal and frontal derivation (left and middle panel) during NREM sleep, or the amount of NREM sleep (right panel) in the 2 h interval after sleep onset. Individuals ($n = 9$) contributed with three values (days 1–3; different symbols). Straight lines depict linear regressions.

either due to the lack of the stimulation of the motor areas or as a consequence of the change in stimulation elicited by other behaviours.

When running was prevented, frontal EEG power was increased in the high delta and low theta frequencies. The frontal predominance was maximal in the dark period following the RW block, diminished in the subsequent light period, and was no longer present when running was again allowed the next day (Figs 2 and 3). These results are consistent with the notion that region-specific changes in neuronal plasticity may lead to enhancement of regional EEG gradients during subsequent sleep; this was supported by the results from the correlation analysis. The intensity of running showed a negative correlation with frontal predominance of SWA in NREM sleep ($r = -0.60$; $P < 0.01$). This means that waking activities other than the stereotyped running elicited the more pronounced regional differences. When the RW was blocked, the mice still showed a large amount of locomotion (Table 1), and the concomitant increase in EEG power in the theta frequencies during locomotion suggests that they were engaged in exploratory behaviour (Huber *et al.*, 1999). Such behaviour, requiring more coordinated locomotion than running, could induce a use-dependent increase in sleep intensity in the motor areas of the frontal cortex. Neuronal plasticity may be involved in mediating the effects of such sensorimotor stimulation on local sleep regulation. Recent data in humans have shown that acquiring skills in a motor task results in a local increase of slow waves during subsequent sleep that correlates with performance improvement (Huber *et al.*, 2004). There is evidence that RW activity is associated with neuronal plasticity in the motor cortex. Thus, cell proliferation was enhanced as a result of running in mice (Ehninger and Kempermann, 2003). Moreover, wheel-running was associated with neuronal plasticity in the hippocampus (van Praag *et al.*, 1999: Farmer *et al*, 2004). It is possible that prolonged vigorous daily running might have changed the corresponding neuronal circuits in the motor

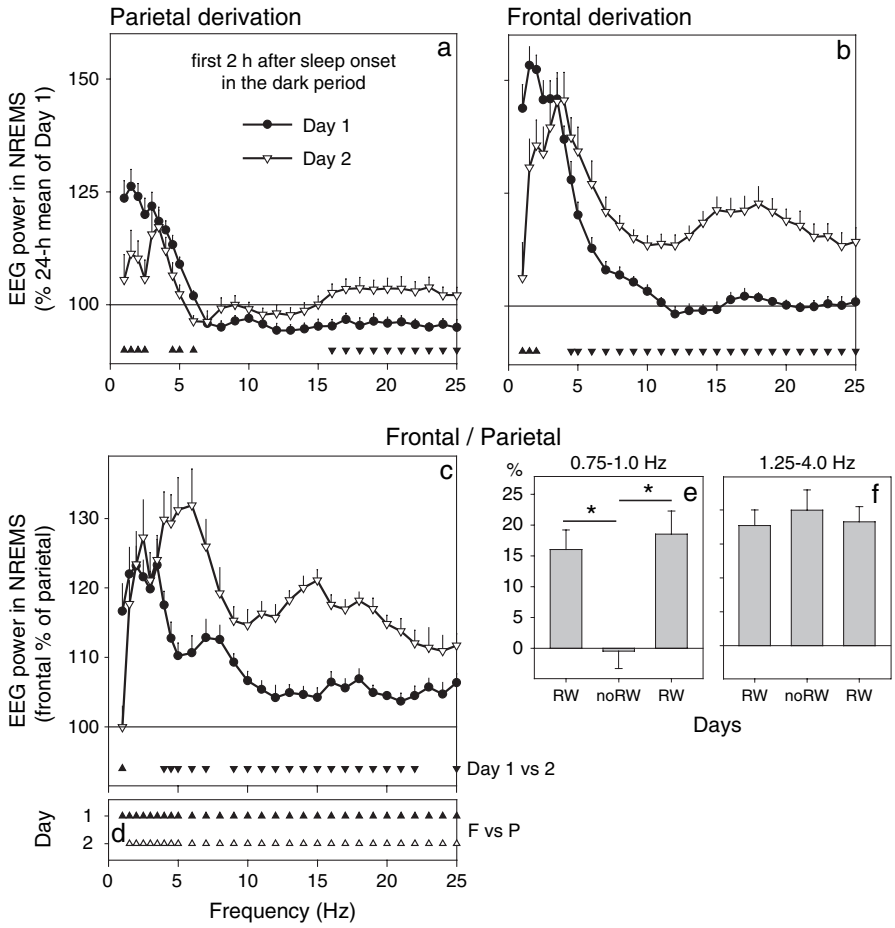

Figure 6. EEG power spectra in NREM sleep in the first 2 h interval after individually determined sleep onset on day 1 and day 2 ($n = 9$). Data are shown for the parietal (upper panel *a*) and frontal derivation (upper panel *b*) of the right hemisphere, and for the ratio between the two derivations (lower panel *c*). Mean values of relative power density of each bin are expressed as percentage of the 24 h mean value in NREM sleep of the same bin on day 1 (= 100%). Triangles below the curves in panel *c* indicate frequency bins where EEG power differed between days (day 1 versus day 2; paired *t*-test, $P < 0.05$). Triangles in left bottom panel (*d*): frequency bins where EEG power in the frontal derivation was above corresponding values in the parietal derivation, separately for day 1 and day 2 (F versus P). Orientation of triangles indicates the direction of deviation. Right bottom panels *e* and *f*: difference in EEG power in NREM sleep between the frontal and parietal derivation during the first 2 h after sleep onset (0 % = no difference). Mean values of EEG power are shown separately for the 0.75–1.0 Hz bin (*e*) and the remaining frequencies of the delta band (1.25–4.0 Hz, *f*) for the three days. RW, with access to the running wheel; noRW, the wheel was blocked. Asterisks denote significant differences between days (corrected for multiple comparison; $P < 0.01$; paired *t*-tests).

cortex. The cessation of running and/or a shift to other types of behaviour on day 2 could therefore affect activity patterns of the neurons in the frontal area resulting in EEG spectral changes during sleep. Similarly, some spectral EEG differences can be expected when comparing mice well adapted to a RW with normally housed mice. The prolonged waking episode elicited by wheel-running led to a prominent increase of SWA during sleep (Fig. 4), qualitatively comparable to the effect of 6 h SD in the same strain of mice housed without a RW (Huber *et al.*, 2000a). Positive correlations between the duration of waking and the amount of NREM sleep, and with the levels of SWA in the first 2 h of sleep, were found. In contrast, the intensity of running had no effect on sleep or the EEG of the motor cortex during sleep. The relationship between the duration of SD or of short epochs of spontaneous waking and the increase of SWA in subsequent NREM sleep is well established in rodents (e.g. Tobler and Borbély, 1986; Strijkstra and Daan, 1998; Huber *et al.*, 1999; Franken *et al.*, 2001; Deboer and Tobler, 2003; Larkin and Heller, 1998, 2003). Our study shows that a similar increase can be elicited by prolonged spontaneous activity, indicating that the SWA increase after SD is not merely a consequence of unspecific effects of the deprivation procedure. A novel finding was that the amount of NREM sleep increases as a function of the duration of preceding spontaneous waking episode. The positive correlation between the duration of preceding waking and SWA, as well as with the amount of sleep, supported the notion that both these variables reflect homeostatic sleep mechanisms.

Invariably delta activity of the frontal derivation exceeded the corresponding values of the parietal derivation, confirming previous studies in rodents (mice: Huber *et al.*, 1999, 2000b; Vyazovskiy *et al.*, 2002, 2004b; rats: Schwierin *et al.*, 1999; Djungarian hamster: Palchykova *et al.*, 2002). Interestingly, only frontal predominance of the low delta frequency bin (0.75–1.0 Hz) was determined by the increase in sleep propensity, and not the 1.25–4.0 Hz band, which remained at the same level on all days (Fig. 7). On day 2 the frontal–parietal ratio of EEG power in this band remained high despite the larger amount of sleep (Fig. 6, Table 1). The higher frontal predominance in the lower delta frequency after longer waking episodes (Fig. 7) has been shown previously in two mouse strains (Huber *et al.*, 2000b) and in humans (Finelli *et al.*, 2001), suggesting that the build-up

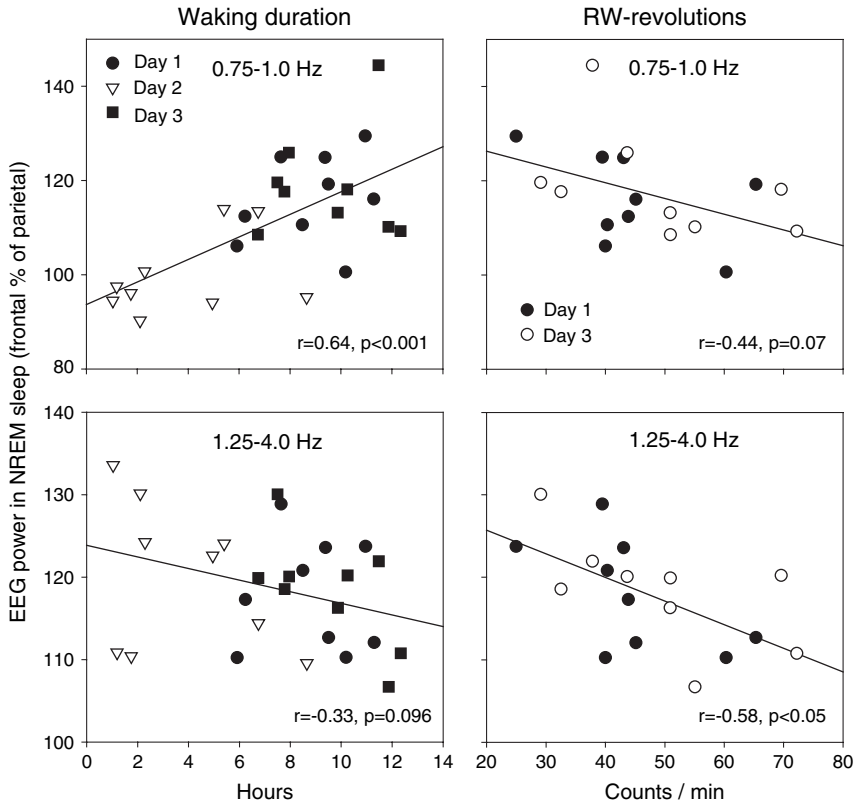

Figure 7. Scatter plots of the correlation between the duration of waking prior to sleep onset on the three days (left panels) or intensity of RW activity (RW revolutions) on days 1 and 3 (right panels) and the frontal–parietal ratio of EEG power in the 0.75–1.0 Hz (upper panels) or 1.25–4.0 Hz band (lower panels) during NREM sleep in the subsequent 2 h. Every individual ($n = 9$) contributed with three (left: day 1, 2, 3) or two values (right, day 1, 3). Straight lines depict linear regressions.

rate during waking in the frontal derivation is faster than in the parietal derivation. Thus local sleep homeostasis is best reflected by low frequency activity in the NREM sleep EEG. The low frequency bin may correspond to the slow cortical oscillation, a typical feature of normal physiological sleep in the cat (Steriade *et al.*, 1993; Amzica and Steriade, 1998; Destexhe *et al.*, 1999) and in humans (Achermann and Borbély, 1997; Steriade and Amzica, 1998).

The mechanisms of the frontal predominance of SWA during sleep are unknown. The regional differences observed in the higher delta frequencies may reflect morphological differences in cortical areas. This interpretation is supported by recent data in mice showing that the frontal predominance of the high delta frequencies under increased sleep pressure is reduced as a result of congenital callosal dysgenesis (Vyazovskiy and Tobler, 2004a, 2005). In these mice callosal fibres fail to cross the midline and instead form a novel structure — the longitudinal bundle of Probst that provides redundant connectivity predominantly in the rostral portion of the neocortex (Ozaki and Shimada, 1988; Ozaki *et al.*, 1989; Ozaki and Wahlsten, 1993). Moreover, a functional component may contribute to the hyperfrontality. Thus, it can be hypothesized that the threshold for entraining a large neuronal population in synchronous firing is lowered in the frontal cortex. This area can be more responsive to activation, as suggested by the faster rate of increase in slow delta activity as a function of prolonged waking (Fig. 7).

There is evidence in rodents for a topographical organization of cortical afferents from the basal forebrain (Lamour *et al.*, 1982), raphe nucleus (Bennett-Clarke *et al.*, 1994), locus coeruleus (Loughlin *et al.*, 1992) and thalamus (Garel and Rubenstein, 2004). This patterning of inputs of different modality and functional specificity could result in a segregation of cortical areas and result in regional differences of the EEG during waking and subsequent sleep.

Running behaviour was characterized by a surge in power within the theta band in the waking EEG, while delta activity was suppressed in both derivations. The increased theta activity during running may be related to the positive correlation between fos expression in the hippocampus and the distance run in the wheel prior to fos measurements (Rhodes *et al.*, 2003). In addition, RW activity in the middle of the dark period has been shown to increase cell proliferation in the hippocampus (Holmes *et al.*, 2004).

Summary and Conclusion

This is the first study in animals addressing the mechanisms of frontal predominance of SWA during sleep. The RW accessibility which elicited spontaneous, monotonous running activity allowed investigating the effects of the change in behavior when the RW was blocked. The increased predominance of delta and low theta EEG power in NREM sleep in the derivation above the frontal motor cortex during the RW block support the hypothesis that local, activity-dependent mechanisms contribute to the regional differences in homeostatic increase of sleep intensity encountered after a period of wakefulness. Moreover, the data suggest that EEG power between 0.75 and

1.0 Hz, corresponding to the slow oscillation described in cats and humans, is related to local sleep homeostasis.

Notes

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